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(54) A test device for performing specific binding assays

Testgerät zur Durchführung von spezifischen Bindungsprüfungen

Dispositif d'analyse pour l'exécution d'essais de liaisons spécifiques

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Description

[0001] This patent results from a divisional of European patent application No. 88303744.2 published as EP 0291194 B1.

[0002] The present invention relates to assays involving specific binding, especially immunoassays.

[0003] In particular, the invention relates to analytical devices which are suitable for use in the home, clinic or doctor's surgery and which are intended to give an analytical result which is rapid and which requires the minimum degree of skill and involvement from the user. The use of test devices in the home to test for pregnancy and fertile period (ovulation) is now commonplace, and a wide variety of test devices and kits are available commercially. Without exception, the commercially-available devices all require the user to perform a sequence of operations before the test result is observable. These operations necessarily involve time, and introduce the possibility of error.

[0004] It is an object of the present invention to provide a test device which is readily usable by an unskilled person and which preferably merely requires that some portion of the device is contacted with the sample (e.g. a urine stream in the case of a pregnancy or ovulation test) and thereafter no further actions are required by the user before an analytical result can be observed. Ideally the analytical result should be observable within a matter of minutes following sample application, e.g. ten minutes or less.

[0005] The use of reagent-impregnated test strips in specific binding assays, such as immunoassays, has previously been proposed. In such procedures a sample is applied to one portion of the test strip and is allowed to permeate through the strip material, usually with the aid of an eluting solvent such as water. In so doing, the sample progresses into or through a detection zone in the test strip wherein a specific binding reagent for an analyte suspected of being in the sample is immobilised. Analyte present in the sample can therefore become bound within the detection zone. The extent to which the analyte becomes bound in that zone can be determined with the aid of labelled reagents which can also be incorporated in the test strip or applied thereto subsequently. Examples of prior-proposals utilising these principles are given in Thyroid Diagnostics Inc GB 1589234, Boots-Celltech Diagnostics Limited EP 0225054, Syntex (USA) Inc EP 0183442, and Behringwerke AG EP 0186799.

[0006] The present invention is concerned with adapting and improving the known techniques, such as those referred to in the above publications, to provide diagnostic test devices especially suitable for home use which are quick and convenient to use and which require the user to perform as few actions as possible.

[0007] The prior European patent application 0 299 428 (relevant with respect to article 54(3) EPC) relates to specific binding assay methods, kits and devices and utilizing chromatographically mobile specific binding reagents labelled with colloidal particles. Specific binding reagents labelled with colloidal particles such as gold may be subjected to rapid chromatographic solvent transport or chromatographic media by means of selected solvents and chromatographic transport facilitating agents. Particular embodiments are illustrated by Figures 4a, 4b and 4c, as well as the description parts in conjunction therewith. According to this, following the removal of a tab, the material to be tested is applied to a second zone of a porous carrier, which is located between a third zone with the reactant immobilized there and a first zone with the labelled specific binding reagent applied there (mobile in the case of liquid adsorption). To render the device operative, the tab must be refitted in order to obtain a closed system. Then the test is started by receiving a chromatographic liquid, which contains no analyte, i.e. not hCG to be tested. The known device has no means enabling the extent (if any) to which a labelled reagent becomes bound in the detection zone to be observed.

[0008] The invention provides an analytical test device comprising a dry porous carrier, unlabelled specific binding reagent for an analyte, which unlabelled reagent is permanently immobilised in a detection zone on the porous carrier and is therefore not mobile in the moist state, and in the dry state upstream from the detection zone a labelled specific binding reagent for the same analyte which labelled specific binding reagent is freely mobile within the porous carrier when in the moist state, such that liquid test sample suspected of containing the analyte and applied to the device can pick up labelled reagent and thereafter permeate into the detection zone, characterized in, that a) the label is a particulate direct label; b) the porous carrier is contained within a hollow casing constructed of moisture-impervious solid material and provided with means enabling the extent (if any) to which the labelled reagent becomes bound in the detection zone to be observed; c) the porous carrier is linked to a porous sample receiver and communicates indirectly with the exterior of the hollow casing via the porous sample receiver to which the liquid test sample can be applied and from which the applied liquid test sample can permeate into the porous carrier; and d) the labelled specific binding reagent is contained within the hollow casing.

[0009] Preferably the device of the invention incorporates a porous solid phase material carrying in a first zone a labelled reagent which is retained in the first zone while the porous material is in the dry state but is free to migrate through the porous material when the porous material is moistened, for example by the application of an aqueous liquid sample suspected of containing the analyte, the porous material carrying in a second zone, which is spatially distinct from the first zone, an unlabelled specific binding reagent having specificity for the analyte, and which is capable of participating with the labelled reagent in either a "sandwich" or a "competition" reaction, the unlabelled specific binding reagent being firmly immobilised on the porous material such that it is not free to migrate when the porous material is

in the moist state.

[0010] The invention also provides an analytical method in which a device as set forth in the proceeding paragraph is contacted with an aqueous liquid sample suspected of containing the analyte, such that the sample permeates by capillary action through the porous solid phase material via the first zone into the second zone and the labelled reagent migrates therewith from the first zone to the second zone, the presence of analyte in the sample being determined by observing the extent (if any) to which the labelled reagent becomes bound in the second zone.

[0011] In one embodiment of the invention, the labelled reagent is a specific binding partner for the analyte. The labelled reagent, the analyte (if present) and the immobilised unlabelled specific binding reagent cooperate together in a "sandwich" reaction. This results in the labelled reagent being bound in the second zone if analyte is present in the sample. The two binding reagents must have specificities for different epitopes on the analyte.

[0012] In another embodiment of the invention, the labelled reagent is either the analyte itself which has been conjugated with a label, or is an analyte analogue, ie a chemical entity having the identical specific binding characteristics as the analyte, and which similarly has been conjugated with a label. In the latter case, it is preferable that the properties of the analyte analogue which influence its solubility or dispersibility in an aqueous liquid sample and its ability to migrate through the moist porous solid phase material should be identical to those of the analyte itself, or at least very closely similar. In this second embodiment, the labelled analyte or analyte analogue will migrate through the porous solid phase material into the second zone and bind with the immobilised reagent. Any analyte present in the sample will compete with the labelled reagent in this binding reaction. Such competition will result in a reduction in the amount of labelled reagent binding in the second zone, and a consequent decrease in the intensity of the signal observed in the second zone in comparison with the signal that is observed in the absence of analyte in the sample.

[0013] An important preferred embodiment of the invention is the selection of nitrocellulose as the carrier material. This has considerable advantage over conventional strip materials, such as paper, because it has a natural ability to bind proteins without requiring prior sensitisation. Specific binding reagents, such as immunoglobulins, can be applied directly to nitrocellulose and immobilised thereon. No chemical treatment is required which might interfere with the essential specific binding activity of the reagent. Unused binding sites on the nitrocellulose can thereafter be blocked using simple materials, such as polyvinylalcohol. Moreover, nitrocellulose is readily available in a range of pore sizes and this facilitates the selection of a carrier material to suit particularly requirements such as sample flow rate.

[0014] Another important preferred embodiment of the invention is the use of so called "direct labels", attached to one of the specific binding reagents. Direct labels such as gold sols and dye sols, are already known per se. They can be used to produce an instant analytical result without the need to add further reagents in order to develop a detectable signal. They are robust and stable and can therefore be used readily in a analytical device which is stored in the dry state. Their release on contact with an aqueous sample can be modulated, for example by the use of soluble glazes.

[0015] An important aspect of the invention is the selection of technical features which enable a direct labelled specific binding reagent to be used in a carrier-based analytical device, e.g. one based on a strip format, to give a quick and clear result. Ideally, the result of the assay should be discernable by eye and to facilitate this, it is necessary for the direct label to become concentrated in the detection zone. To achieve this, the direct labelled reagent should be transportable easily and rapidly by the developing liquid. Furthermore, it is preferable that the whole of the developing sample liquid is directed through a comparatively small detection zone in order that the probability of an observable result being obtained in increased.

[0016] Another important aspect of the invention is the use of a directly labelled specific binding reagent on a carrier material comprising nitrocellulose. Preferably the nitrocellulose has a pore size of at least one micron. Preferably the nitrocellulose has a pore size not greater than about 20 microns. In a particularly preferred embodiment, the direct label is a coloured latex particle of spherical or near-spherical shape and having a maximum diameter of not greater than about 0.5 micron. An ideal size range for such particles is from about 0.05 to about 0.5 microns.

[0017] According to the present invention, the porous solid phase carrier is linked to a porous sample receiver to which the liquid sample can be applied and from which the sample can permeate into the porous solid phase carrier. Preferably, the porous solid phase carrier is contained within a moisture-impermeable casing or housing and the porous sample receiver, with which the porous solid phase carrier is linked, extends out of the housing and can act as a means for permitting a liquid sample to enter the housing and permeate the porous solid phase carrier. The housing should be provided with means, e.g. appropriately placed apertures, which enable the second zone of the porous solid phase carrier (carrying the immobilised unlabelled specific binding reagent) to be observable from outside the housing so that the result of the assay can be observed. If desired, the housing may also be provided with further means which enable a further zone of the porous solid phase carrier to be observed from outside the housing and which further zone incorporates control reagents which enable an indication to be given as to whether the assay procedure has been completed. Preferably the housing is provided with a removable cap or shroud which can protect the protruding porous sample receiver during storage before use. If desired, the cap or shroud can be replaced over the protruding porous sample receiver, after sample application, while the assay procedure is being performed.

[0018] An important embodiment of the invention is a pregnancy testing device comprising a hollow elongated casing

containing a dry porous nitrocellulose carrier which communicates indirectly with the exterior of the casing via a bibulous urine sample receiver which protrudes from the casing and which can act as a reservoir from which urine is released into the porous carrier, the carrier containing in a first zone a highly-specific anti-hCG antibody bearing a coloured "direct" label, the labelled antibody being freely mobile within the porous carrier when in the moist state, and in a second zone spatially distinct from the first zone an highly-specific unlabelled anti-hCG antibody which is permanently immobilised on the carrier material and is therefore not mobile in the moist state, the labelled and unlabelled antibodies having specificities for different hCG epitopes, the two zones being arranged such that a urine sample applied to the porous carrier can permeate via the first zone into the second zone, and the casing being constructed of opaque or translucent material incorporating at least one aperture through which the analytical result may be observed, together with a removable and replaceable cover for the protruding bibulous urine sample receiver. A fertile period prediction device, essentially as just defined except that the analyte is LH, is an important alternative.

[0019] Such devices can be provided as kits suitable for home use, comprising a plurality (e.g. two) of devices individually wrapped in moisture impervious wrapping and packaged together with appropriate instructions to the user.

[0020] The porous sample receiver can be made from any bibulous, porous or fibrous material capable of absorbing liquid rapidly. The porosity of the material can be unidirectional (ie with pores or fibres running wholly or predominantly parallel to an axis of the receiver or multidirectional (omnidirectional, so that the member has an amorphous sponge-like structure). Porous plastics material, such as polypropylene, polyethylene (preferably of very high molecular weight), polyvinylidene flouride, ethylene vinylacetate, acrylonitrile and polytetrafluoro-ethylene can be used. It can be advantageous to pre-treat the porous sample receiver with a surface-active agent during manufacture, as this can reduce any inherent hydrophobicity in the receiver and therefore enhance its ability to take up and deliver a moist sample rapidly and efficiently. Porous sample receivers can also be made from paper or other cellulosic materials, such as nitro-cellulose. Materials that are now used in the nibs of so-called fibre tipped pens are particularly suitable and such materials can be shaped or extruded in a variety of lengths and cross-sections appropriate in the context of the invention. Preferably the material comprising the porous sample receiver should be chosen such that it can be saturated with aqueous liquid within a matter of seconds. Preferably the material remains robust when moist, and for this reason paper and similar materials are less preferred in any embodiment wherein the porous sample receiver protrudes from a housing. The liquid must thereafter permeate freely from the porous sample receiver into the porous solid phase material.

[0021] If present, the "control" zone can be designed merely to convey an unrelated signal to the user that the device has worked. For example, the control zone can be loaded with an antibody that will bind to the labelled antibody from the first zone, e.g. an "anti-mouse" antibody if the labelled body is one that has been derived using a murine hybridoma, to confirm that the sample has permeated the test strip. Alternatively, the control zone can contain an anhydrous reagent that, when moistened, produces a colour change or colour formation, e.g. anhydrous copper sulphate which will turn blue when moistened by an aqueous sample. As a further alternative, a control zone could contain immobilised analyte which will react with excess labelled reagent from the first zone. As the purpose of the control zone is to indicate to the user that the test has been completed, the control zone should be located downstream from the second zone in which the desired test result is recorded. A positive control indicator therefore tells the user that the sample has permeated the required distance through the test device.

[0022] The label is a direct label, ie an entity which, in its natural state, is readily visible either to the naked eye, or with the aid of an optical filter and/or applied stimulation, e.g. UV light to promote fluorescence. For example, minute coloured particles, such as dye sols, metallic sols (e.g. gold), and coloured latex particles, are very suitable. Of these options, coloured latex particles are most preferred. Concentration of the label into a small zone or volume should give rise to a readily detectable signal, e.g. a strongly-coloured area. This can be evaluated by eye, or by instruments if desired.

[0023] Coupling of the label to the specific binding reagent can be by covalent bonding, if desired, or by hydrophobic bonding. Such techniques are commonplace in the art, and form no part of the present invention. In the preferred embodiment, where the label is a direct label such as a coloured latex particle, hydrophobic bonding is preferred.

[0024] In all embodiments of the invention, it is essential that the labelled reagent migrates with the liquid sample as this progresses to the detection zone. Preferably, the flow of sample continues beyond the detection zone and sufficient sample is applied to the porous material in order that this may occur and that any excess labelled reagent from the first zone which does not participate in any binding reaction in the second zone is flushed away from the detection zone by this continuing flow. If desired, an absorbant "sink" can be provided at the distal end of the carrier material. The absorbent sink may comprise of, for example, Whatman 3MM chromatography paper, and should provide sufficient absorptive capacity to allow any unbound conjugate to wash out of the detection zone. As an alternative to such a sink it can be sufficient to have a length of porous solid phase material which extends beyond the detection zone.

[0025] The presence or intensity of the signal from the label which becomes bound in the second zone can provide a qualitative or quantitative measurement of analyte in the sample. A plurality of detection zones arranged in series on the porous solid phase material, through which the aqueous liquid sample can pass progressively, can also be used

to provide a quantitative measurement of the analyte, or can be loaded individually with different specific binding agents to provide a multi-analyte test.

[0026] The immobilised specific binding reagent in the second zone is preferably a highly specific antibody, and more preferably a monoclonal antibody. In the embodiment of the invention involving the sandwich reaction, the labelled reagent is also preferably a highly specific antibody, and more preferably a monoclonal antibody.

[0027] Preferably the carrier material is in the form of a strip or sheet to which the reagents are applied in spacially distinct zones, and the liquid sample is allowed to permeate through the sheet or strip from one side or end to another.

[0028] If desired, a device according to the invention can incorporate two or more discrete bodies of porous solid phase material, e.g. separate strips or sheets, each carrying mobile and immobilised reagents. These discrete bodies can be arranged in parallel, for example, such that a single application of liquid sample to the device initiates sample flow in the discrete bodies simultaneously. The-separate analytical results that can be determined in this way can be used as control results, or if different reagents are used on the different carriers, the simultaneous determination of a plurality of analytes in a single sample can be made. Alternatively, multiple samples can be applied individually to an array of carriers and analysed simultaneously.

[0029] The material comprising the porous solid phase is preferably nitrocellulose. This has the advantage that the antibody in the second zone can be immobilised firmly without prior chemical treatment. If the porous solid phase material comprises paper, for example, the immobilisation of the antibody in the second zone needs to be performed by chemical coupling using, for example, CNBr, carbonyldiimidazole, or tresyl chloride.

[0030] Following the application of the antibody to the detection zone, the remainder of the porous solid phase material should be treated to block any remaining binding sites elsewhere. Blocking can be achieved by treatment with protein (e.g. bovine serum albumin or milk protein), or with polyvinylalcohol or ethanolamine, or any combination of these agents, for example. The labelled reagent for the first zone can then be dispensed onto the dry carrier and will become mobile in the carrier when in the moist state. Between each of these various process steps (sensitisation, application of unlabelled reagent, blocking and application of the labelled reagent), the porous solid phase material should be dried.

[0031] To assist the free mobility of the labelled reagent when the porous carrier is moistened with the sample, it is preferable for the labelled reagent to be applied to the carrier as a surface layer, rather than being impregnated in the thickness of the carrier. This can minimise interaction between the carrier material and the labelled reagent. In a preferred embodiment of the invention, the carrier is pre-treated with a glazing material in the region to which the labelled reagent is to be applied. Glazing can be achieved, for example, by depositing an aqueous sugar or cellulose solution, e.g. of sucrose or lactose, on the carrier at the relevant portion, and drying. The labelled reagent can then be applied to the glazed portion. The remainder of the carrier material should not be glazed.

[0032] Preferably the porous solid phase material is nitrocellulose sheet having a pore size of at least about 1 micron, even more preferably of greater than about 5 microns, and yet more preferably about 8-12 microns. Very suitable nitrocellulose sheet having a nominal pore size of up to approximately 12 microns, is available commercially from Schleicher and Schuell GmbH.

[0033] Preferably, the nitrocellulose sheet is "backed", e.g. with plastics sheet, to increase its handling strenth. This can be manufactured easily by forming a thin layer of nitrocellulose on a sheet of backing material. The actual pore size of the nitrocellulose when backed in this manner will tend to be, lower than that of the corresponding unbacked material.

[0034] Alternatively, a pre-formed sheet of nitrocellulose can be tightly sandwiched between two supporting sheets of solid material, e.g. plastics sheets.

[0035] It is preferable that the flow rate of an aqueous sample through the porous solid phase material should be such that in the untreated material, aqueous liquid migrates at a rate of 1cm in not more than 2 minutes, but slower flow rates can be used if desired.

[0036] The spatial separation between the zones, and the flow rate characteristics of the porous carrier material, can be selected to allow adequate reaction times during which the necessary specific binding can occur, and to allow the labelled reagent in the first zone to disperse in the liquid sample and migrate through the carrier. Further control over these parameters can be achieved by the incorporation of viscosity modifiers (e.g. sugars and modified celluloses) in the sample to slow down the reagent migration.

[0037] Preferably, the immobilised reagent in the second zone is impregnated throughout the thickness of the carrier in the second zone (e.g. throughout the thickness of the sheet or strip if the carrier is in this form). Such impregnation can enhance the extent to which the immobilised reagent can capture any analyte present in the migrating sample.

[0038] The reagents can be applied to the carrier material in a variety of ways. Various "printing" techniques have previously been proposed for application of liquid reagents to carriers, e.g. micro-syringes, pens using metered pumps, direct printing and ink-jet printing, and any of these techniques can be used in the present context. To facilitate manufacture, the carrier (e.g. sheet) can be treated with the reagents and then subdivided into smaller portions (e.g. small narrow strips each embodying the required reagent-containing zones) to provide a plurality of identical carrier units.

[0039] By way of example only, some preferred embodiments of the invention will now be described in detail with reference to the accompanying drawings.

Embodiment 1

[0040] Figure 1 of the accompanying drawings represents an isometric view of an assay device in accordance with the invention, and Figure 2 represents a cross-sectional side elevation of the device shown in Figure 1.

[0041] Referring to Figure 1, the device comprises a housing or casing 500 of elongate rectangular form having at one end 501 a portion 502 of reduced cross-sectional area. A cap 503 can be fitted onto portion 502 and can abut against the shoulder 504 at end 501 of the housing. Cap 503 is shown separated from housing 500. Extending beyond end 505 of portion 502 is a porous member 506. When cap 503 is fitted onto portion 502 of the housing, it covers porous sample receiver 506. Upper face 507 of housing 500 incorporates two apertures 508 and 509.

[0042] Referring to Figure 2, it can be seen that housing 500 is of hollow construction. Porous sample receiver 506 extends into housing 500 and contacts a strip of porous carrier material 510. Porous sample receiver 506 and strip 510 overlap to ensure that there is adequate contact between these two materials and that a liquid sample applied to member 506 can permeate sample receiver 506 and progress into strip 510. Strip 510 extends further into housing 500. Strip 510 is "backed" by a supporting strip 511 formed of transparent moisture-impermeable plastics material. Strip 510 extends beyond apertures 508 and 509. Means are provided within housing 500 by webbs 512 and 513 to hold strip 510 firmly in place. In this respect, the internal constructional details of the housing are not a significant aspect of the invention as long as the strip is held firmly in place within the housing, and porous member 506 is firmly retained in the housing and adequate fluid permeable contact is maintained between sample receiver 506 and strip 510. The transparent backing strip 511 lies between strip 510 and apertures 508 and 509 and can act as a seal against ingress of moisture from outside the housing 500 via these apertures. If desired, the residual space 514 within the housing can contain moisture-absorbant material, such as silica gel, to help maintain the strip 510 in the dry state during storage. The reagent-containing zones in strip 510 are not depicted in Figure 1, but the first zone containing the labelled reagent which is mobile when the strip is moistened will lie in the region between the porous sample receiver 506 and aperture 508. The second zone containing the immobilised unlabelled reagent will lie in the region exposed through aperture 508 in order that when the device has been used in an assay, the result can be observed through aperture 508. Aperture 509 provides means through which a control zone containing further reagents which may enable the adequate permeation of sample through the strip to be observed.

[0043] In operation, the protective cap 503 is removed from the holder and sample receiver 506 is exposed to a liquid sample e.g. by being placed in a urine stream in the case of a pregnancy test. After exposing sample receiver 506 to the liquid sample for a time sufficient to ensure that sample receiver 506 is saturated with the sample, the cap 503 can be replaced and the device placed aside by the user for an appropriate period time (e.g. two or three minutes) while the sample permeates test strip 510 to provide the analytical result. After the appropriate time, the user can observe the test strip through apertures 508 and 509 and can ascertain whether the assay has been completed by observing the control zone through aperture 509, and can ascertain the result of the assay by observing the second zone through aperture 508.

[0044] During manufacture, the device can be readily assembled from, for example, plastics material with the housing 500 being moulded in two parts (e.g. upper and lower halves 515 and 516) which can be securely fastened together (e.g. by ultrasonic welding) after the porous member and test strip have been placed within one of the halves and then sandwiched between the two halves. The act of forming this sandwich construction can be used to "crimp" the porous member and test strip together to ensure adequate contact between them. Cap 503 can be moulded as a separate complete item. If desired, apertures 508 and 509 can be provided with transparent inserts which may insure greater security against ingress of extraneous moisture from outside the housing. By providing a tight fit between the end 505 of housing 500 and the protruding porous sample receiver 506, the application of sample to the protruding member will not result in sample entering the device directly and by-passing sample receiver 506. sample receiver 506 therefore provides the sole route of access for the sample to the strip within the housing, and can deliver sample to the strip in a controlled manner. The device as a whole therefore combines the functions of samples and analyser.

[0045] By using the test strip materials and reagents as hereinafter described, a device in accordance with Figures 1 and 2 can be produced which is eminently suitable for use as a pregnancy test kit or fertile period test kit for use in the home or clinic. The user merely needs to apply a urine sample to the exposed porous member and then (after optionally replacing the cap) can observe the test result through aperture 508 within a matter of a few minutes.

[0046] Although described with particular reference to pregnancy tests and fertile period tests, it will be appreciated that the device, as just described, can be used to determine the presence of a very wide variety of analytes if appropriate reagents are incorporated in the test strip. It will be further appreciated that aperture 509 is redundant and may be omitted if the test strip does not contain any control means. Further, the general shape of the housing and cap, both in terms of their length, cross-section and other physical features, can be the subject of considerable variation without

departing from the spirit of the invention.

[0047] A further option is the omission of the labelled reagent from the test strip, this reagent being added to the sample prior to application of the sample to the test device. Alternatively, the labelled reagent can be contained in the protruding porous member 506.

[0048] Figure 3 of the accompanying drawings shows an enlarged view of the porous receiving member and test strip in the device illustrated in Figures 1 and 2.

[0049] The porous sample receiver 506 is linked to the porous test strip 510, backed by the transparent plastics sheet 511, such that liquid can flow in the direction shown by the arrows through the porous sample receiver and into the porous strip. Test zone 517 incorporates the immobilised specific binding reagent, and control zone 518 contains a reagent to indicate that the sample has permeated a sufficient distance along the test strip. A portion of the test strip surface opposite the backing strip 511 and adjacent the porous receiving member 506, carries a glaze 519 on which is deposited a layer 520 of labelled specific binding reagent. The thickness of these two layers as depicted in Figure 3 is grossly exaggerated purely for the purpose of illustration. It will be appreciated that, in practice, the glaze may not form a true surface layer and the glazing material will penetrate the thickness of the strip to some extent. Similarly, the subsequently applied labelled reagent may also penetrate the strip. Nevertheless, the essential objective of reducing any interaction between the labelled reagent and the carrier material forming the strip will be achieved. An aqueous sample deposited in sample receiver 506 can flow therefrom along the length of strip 510 and in so doing, will dissolve glaze 519 and mobilise the labelled reagent, and carry the labelled reagent along the strip and through zone 517.

Embodiment 2

[0050] Figures 4 and 5 illustrate another embodiment of the invention, which is seen in plan view in Figure 4 and in cross-section in Figure 4, the cross-section being an elevation on the line A seen in Figure 4.

[0051] Referring to Figure 4, the test device comprises a flat rectangular casing 600 incorporating a centrally disposed rectangular aperture 601, adjacent the left hand end 602, and two further apertures 603 and 604 near the mid point of the device and arranged such that apertures 601, 603 and 604 lie on the central longitudinal axis of the device corresponding to line A. Although all three apertures are illustrated as being rectangular, their actual shape is not critical.

[0052] Referring to the cross-section seen in Figure 5, the device is hollow and incorporates within it a porous sample receiver adjacent end 602 of casing 600 and lying directly beneath aperture 601. A test strip of similar construction to that described with reference to Embodiment 4, comprising a porous strip 606 backed by a transparent plastics sheet 607 is also contained within casing 600, and extends from the porous receiving member 602, with which the porous carrier is in liquid permeable contact, to the extreme other end of the casing. The transparent backing sheet 607 is in firm contact with the upper inner surface 608 of casing 600, and provides a seal against apertures 603 and 604 to prevent ingress of moisture or sample into the casing. Although not shown in the drawings, the porous test strip 606 will incorporate a labelled specific binding reagent, and a test zone and a control zone placed appropriately in relation to apertures 603 and 604, in a manner analagous to that described in Embodiment 1.

[0053] In operation, an aqueous sample can be applied through aperture 601, e.g. by means of a syringe, to saturate porous sample receiver 605. Thereafter, the aqueous sample can permeate the test strip and after an appropriate time the test result can be observed through apertures 603 and 604.

1. Selection of Liquid Conductive Material

[0054] Representative examples of liquid conductive materials include paper, nitrocellulose and nylon membranes. Essential features of the material are its ability to bind protein; speed of liquid conduction; and, if necessary after pretreatment, its ability to allow the passage of labelled antibodies along the strip. It this is a direct label, it may be desirable for the material to allow flow of particles of size up to a few microns (usually less than 0.5μ). Examples of flow rates obtained with various materials are given below:

	Pore size	Time to Flow 45mm (minutes)
Schleicher + Schuell nitrocellulose (unbacked)	3μ	3.40
	5μ	3.30
	8μ	3.00
	12μ	2.20
polyester-backed	8μ (nominal)	3.40
Whatman Nitrocellulose	5	19.20

(continued)

	Pore size	Time to Flow 45mm (minutes)
Pall "Immunodyne" (nylon)	3	4.00
	5	3.20

[0055] The speed of a test procedure will be determined by the flow rate of the material employed and while any of the above materials can be used some will give faster tests than others.

[0056] Nitrocellulose had the advantage of requiring no activation and will immobilise proteins strongly by absorbtion. "Immunodyne" is pre-activated and requires no chemical treatment. Papers, such as Whatman 3MM, require chemical activation with for example carbonyldiimidazole in order to successfully immobilise antibody.

2. Labels

Preparation of Labels

[0057] A selection of labels which may be used are described below. This list is not exhaustive.

A) Gold Sol Preparation

[0058] Gold sols may be prepared for use in immunoassay from commercially-available colloidal gold, and an antibody preparation such as anti-alpha human chorionic gonadotrophin. Metallic sol labels are described, for example, in European patent specification No. EP 7654.

[0059] For example, colloidal gold G20 (20nm particle size, supplied by Janssen Life Sciences Products) is adjusted to pH 7 with 0.22µ filtered 0.1M K_2CO_3, and 20mls is added to a clean glass beaker. 200µl of anti-alpha hCG antibody, prepared in 2mM borax buffer pH9 at 1mg/ml, and 0.22µ filtered, is added to the gold sol, and the mixture stirred continuously for two minutes. 0.1M K_2CO_3 is used to adjust the pH of the antibody gold sol mixture to 9, and 2mls of 10% (w/v) BSA is added.

[0060] The antibody-gold is purified in a series of three centrifugation steps at 12000g, 30 minutes, and 4°C, with only the loose part of the pellet being resuspended for further use. The final pellet is resuspended in 1% (w/v) BSA in 20mM Tris, 150mM NaC1-pH 8.2.

B) Dye Sol Preparation

[0061] Dye sols (see, for example, European patent specitication No. EP 32270) may be prepared from commercially-available hydrophobic dyestuffs such as Foron Blue SRP (Sandoz) and Resolin Blue BBLS (Bayer). For example, fifty grammes of dye is dispersed in 1 litre of distilled water by mixing on a magnetic stirrer for 2-3 minutes. Fractionation of the dye dispersion can be performed by an initial centrifugation step at 1500g for 10 minutes at room temperature to remove larger sol particles as a solid pellet, with the supernatant suspension being retained for further centrifugation.

[0062] The suspension is centrifuged at 3000g for 10 minutes at room temperature, the supernatant being discarded and the pellet resuspended in 500mls distilled water. This procedure is repeated a further three times, with the final pellet being resuspended in 100mls distilled water.

[0063] The spectra of dye sols prepared as described above can be measured, giving lambda-max values of approximately 657nm for Foron Blue, and 690nm for Resolin Blue. The absorbance at lambda-max, for 1cm path length, is used as an arbitrary measure of the dye sol concentration.

C) Coloured Particles

[0064] Latex (polymer) particles for use in immunoassays are available commerically. These can be based on a range of synthetic polymers, such as polystyrene, polyvinyltoluene, polystyrene-acrylic acid and polyacrolein. The monomers used are normally water-insuluble, and are emulsified in aqueous surfactant so that monomer mycelles are formed, which are then induced to polymerise by the addition of initiator to the emulsion. Substantially sperical polymer particles are produced.

[0065] Coloured latex particles can be produced either by incorporating a suitable dye, such as anthraquinone, in the emulsion before polymerisation, or by colouring the pre-formed particles. In the latter route, the dye should be dissolved in a water-immiscible solvent, such a chroroform, which is then added to an aqueous suspension of the latex particles. The particles take up the non-aqueous solvent and the dye, and can then be dried.

[0066] Preferably such latex particles have a maximum dimension of less than about 0.5 micron.

[0067] Coloured latex particles may be sensitised with protein, and in particular antibody, to provide reagents for use in immunoassays. For example, polystyrene beads of about 0.3 micron diameter, (supplied by Polymer Laboratories) may be sensitised with anti-alpha human chorionic gonadotrophin, in the process described below:

[0068] 0.5ml (12.5mg solids) of suspension is diluted with 1ml of 0.1M borate buffer pH 8.5 in an Eppendorf vial. These particles are washed four times in borate buffer, each wash consisting of centrifugation for 3 minutes at 13000 rpm in an MSE microcentrifuge at room temperature. The final pellet is resuspended in 1ml borate buffer, mixed with 300µg of anti-alpha hCG antibody, and the suspension is rotated end-over-end for 16-20 hours at room temperature. The antibody-latex suspension is centrifuged for 5 minutes at 13000rpm, the supernatant is discarded and the pellet resuspended in 1.5mls borate buffer containing 0.5 milligrammes bovine serum albumin. Following rotation end-over-end for 30 minutes at room temperature, the suspension is washed three times in 5mg/ml BSA in phosphate buffered saline pH7.2, by centrifugation at 13000 rpm for 5 minutes. The pellet is resuspended in 5mg/ml BSA/5% (w/v) glycerol in phosphate buffered saline pH 7.2 and stored at 4°C until used.

(A) Anti-hCG - Dye Sol Preparation

[0069] Protein may be coupled to dye sol in a process involving passive adsorption. The protein may, for example, be an antibody preparation such as anti-alpha human chorionic gonadotrophin prepared in phosphate buffered saline pH 7.4 at 2 milligram/ml. A reaction mixture is prepared which contains 100µl antibody solution, 2mls dye sol, 2mls 0.1M phosphate buffer pH 5.8 and 15.9mls distilled water. After gentle mixing of this solution, the preparation is left for fifteen minutes at room temperature. Excess binding sites may be blocked by the addition of, for example, bovine serum albumin: 4mls of 150mg/ml BSA in 5mM NaCl pH 7.4 is added to the reaction mixture, and after 15 minutes incubation at room temperature, the solution is centrifuged at 3000g for 10 minutes, and the pellet resuspended in 10mls of 0.25% (w/v) dextran/0.5% (w/v) lactose in 0.04M phosphate buffer. This antibody-dye sol conjugate is best stored in a freeze dried form.

(B) LH - Dye Sol Preparation

[0070] Due to the structural homology between the alpha subunits of hCG and LH, alpha hCG antibody can be used to detect LH in a cross-reactive immunoassay. Thus, a labelled antibody may be prepared for use in an LH assay in an identical manner to that described in Example 1, using anti-alpha hCG antibody.

3. Preparation of Reagent Strip

Zonal Impregnation of Liquid-conductive Materials

[0071] Liquid-conducting material with a restricted zone of immobilised protein, particularly antibody, can be prepared for example as follows:

[0072] A rectangular sheet of Schleicher and Schuell backed 8µ nitrocellulose measuring 25cm in length and 20cm in width may have a reaction zone formed upon it by applying a line of material about 1mm wide at 5cm intervals along its length and extending throughout its 20cm width. The material can, for example, be a suitably selected antibody preparation such as anti-beta (human chorionic gonadotropin) of affinity Ka at 10^9, prepared in phosphate buffered saline pH 7.4 at 2 milligram/ml, suitable for immunoassay of human chorionic gonadotrophin using a second (labelled) anti-hCG antibody in a sandwich format. This solution can be deposited by means of a microprocessor-controlled microsyringe, which delivers precise volumes of reagent through a nozzle, preferably 2mm diameter. When the applied material has been allowed to dry for 1 hour at room temperature, excess binding sites on the nitrocellulose are blocked with an inert compound such as polyvinyl alcohol (1% w/v in 20mM Tris pH 7.4) for 30 minutes at room temperature, and sheets are thoroughly rinsed with distilled water prior to drying for 30 minutes at 30°C.

[0073] In one embodiment, the liquid conductive material can then be cut up into numerous strips 5cm in length and 1cm in width, each strip carrying a limited zone of the immobilised antibody to function as an immunosorbent part way (e.g. about half way) along its length. In this example the test strip is used with a liquid label which is mixed with sample. In use, this limited zone then becomes a test reaction zone in which the immunoassay reactions take place.

[0074] In another embodiment, the label may be dispensed/deposited into/on a restricted zone before cutting up the liquid-conductive material into strips. By way of example, this reagent may be dye sol or dye polymer-conjugated anti-hCG antibody prepared as described under dye sol preparation, said reagent being retained in the zone when the material is in the dry state but which is free to migrate through the carrier material when the material is moistened, for example, by the application of liquid sample containing the analyte to be determined. This mobile reagent zone is applied, for example, as follows:

[0075] A sheet of Schleicher and Schuell backed 8µ nitrocellulose, 25cm in length and 20cm in width with zones of immobilised antibody at 5cm intervals along its length, is prepared as described previously. Prior to the deposition of dye labelled antibody, a sublayer of, for example, 60% w/v of sucrose in distilled water is applied by airbrush on the microprocessor controlled system at 6cm intervals along the length of the sheet. Then several passes (e.g. three) of dye labelled antibody prepared in 1% methacel KAM (Trademark for methylcellulose from Dow Chemical Company) and 0.6% (w/v) polyvinylalcohol are applied by airbrush or by microsyringe directly on top of the sublayer. Sheets are then allowed to dry, and cut into strips 5cm in length and 1cm in width, to be used in the completed device.

[0076] Gold sols, or coloured polystyrene particles can be deposited by a similar process.

[0077] In addition to the test zone various control zone options can be operated. For example a zone of anti-species IgG may be deposited after the test zone.

4. Sandwich Assays Using Strip Format

[0078] A sandwich-type reaction may be performed for the detection of human chorionic gonadotrophin (hCG) in a liquid sample. Preferably the label used is a direct label which is readily visible to the naked eye. Dye sols, gold sols or coloured latex particles may be linked to anti hCG antibody, as described above.

[0079] With direct labels, assays may be performed in which fresh urine samples are applied directly from the urine stream, or by delivering an appropriate volume (e.g. 100µl) from a container using a pipette to the absorbent wick of the test device. Each sample is allowed to run for five minutes in the device, and the colour generated at the reactive zone read either by eye, or using a light reflectometer.

[0080] A similar embodiment can be prepared using lutenising hormone (LH) instead of hCG.

5. Competitive Assays

[0081] A competitive type assay may be performed as exemplified by estrone-3-glucuronide, a urinary metabolite of estrone. Conjugates of estrone-3-glucuronide and bovine serum albumin are prepared as follows:

Preparation of BSA - Estrone-3-glucuronide

[0082] The conjugation of E-3-G and BSA may be achieved through the use of a mixed anhydride. All of the glassware, solvents and reagents employed in the preparation of the activated species must be thoroughly dried using an oven, dessicator or molecular sieves, as appropriate, for at least 24 hours.

[0083] Solutions of E-3-G (2nM) in dry dimethylformamide (DMF) and tri-n-butylamine (TnB) (10nM) in dry DMF were equilibrated separately at 4°C. Using pre-cooled glassware E-3-G in DMF (1.25ml) and TnB in DMF (0.25ml) were added to a pre-cooled 5ml Reactivial containing a magnetic stirrer. A solution of isobutyl chloroformate in dry DMF (10nM) was prepared and an aliquot (0.25ml) was cooled to 4°C and added to the Reactivial. The contents of the Reactivial were stirred for 20 minutes at 4°C and a solution of BSA (1mg/ml) in bicarbonate buffer (0.5%) was prepared. When the mixed anhydride incubation was complete, the contents of the Reactivial were added to the BSA solution (2.5ml) and stirred on a magnetic stirrer for 4 hours at 4°C. The conjugate preparation was purified by passage through a Tris buffer equilibrated Pharmacia PD-10 Sephadex G-25 column, transferred to an amber glass storage bottle and stored at 4°C.

Preparation of BSA - E-3-G dye Sol

[0084] A dispersion of dye (5% w/v) in distilled water was prepared with thorough mixing and aliquots were centrifuged at 3850rpm (1500g) for 10 minutes in a bench top centrifuge. The pellet was discarded and the supernatant was retained and centrifuged in aliquots at 4850rpm (3000g) for 10 minutes in a bench top centrifuge. The supernatant was discarded and the pellet was resuspended in half of its original volume in distilled water. This step was repeated four times to wash the pellet. The pellet was finally resuspended in distilled water and the absorbance at lambda max was determined.

[0085] Solutions of dye sol in distilled water and E-3-G/BSA conjugate diluted in phosphate buffer were mixed to give final concentrations of 10µg/ml conjugate (based on BSA content) and an extrapolated dye sol optical density of 20 at the absorbance maximum. The reaction mixture was incubated for 15 minutes at room temperature and blocked for 15 minutes at room temperature with BSA in a NaCl solution (5mM, pH7.4) to yield a final BSA concentration of 25mg/ml. The reaction mixture was centrifuged at 4850rpm (3000g) for 10 minutes in a benchtop centrifuge, the supernatant was discarded and the pellet was resuspended in half of its original volume in Dextran (0.25% w/v)/Lactose (0.5% w/v) phosphate (0.04M pH5.8) buffer.

Preparation of E-3-G Test Strips

[0086] Antibodies to E-3-G were deposited as described in example 3. BSA - E-3-G dye sol was deposited on the strips as described in 3.

Determination of E-3-G

[0087] Using reagents described above, a standard curve can be generated by running strips with samples with known concentrations of E-3-G. The colour at the immobile zone can be read, for example using a Minolta chromameter, and the concentration of E-3-G calculated by extrapolating from the reflectance value.

Claims

1. An analytical test device comprising a dry porous carrier (510), unlabelled specific binding reagent for an analyte which unlabelled reagent is permanently immobilised in a detection zone (517) on the porous carrier and is therefore not mobile in the moist state, and in the dry state upstream from the detection zone a labelled specific binding reagent for the same analyte which labelled specific binding reagent is freely mobile within the porous carrier when in the moist state, such that liquid test sample suspected of containing the analyte and applied to the device can pick up labelled reagent and thereafter permeate into the detection zone (517), **characterised in that**

   a) the label is a particulate direct label;
   b) the porous carrier is contained within a hollow casing (500) constructed of moisture-impervious solid material and provided with means (508) enabling the extent (if any) to which the labelled reagent becomes bound in the detection zone (517) to be observed;
   c) the porous carrier is linked to a porous sample receiver (506) and communicates indirectly with the exterior of the hollow casing via the porous sample receiver to which the liquid test sample can be applied and from which the applied liquid test sample can permeate into the porous carrier; and
   d) the labeled specific binding reagent is contained within the hollow casing (500).

2. A test device according to claim 1, **characterised in that** the porous sample receiver is made from material having unidirectional or multidirectional porosity, preferably porous plastics material.

3. A test device according to claim 1 or claim 2, **characterised in that** the particulate direct label is a dye sol or a gold sol.

4. A test device according to claim 1 or claim 2, **characterised in that** the particulate direct label is coloured latex particles having a maximum diameter of not greater than about 0.5 µm.

5. A test device according to any one of the preceding claims, **characterised in that** the hollow casing is constructed of opaque or translucent material.

6. A test device according to any one of the preceding claims, **characterised in that** the hollow casing is moulded from plastics material.

7. A test device according to any one of the preceding claims, **characterised in that** the porous carrier comprises a strip or sheet of porous material.

8. A test device according to claim 7, **characterised in that** the porous carrier comprises a strip or sheet of porous material backed with a layer (511) of transparent moisture-impervious material, the transparent layer being in contact with the inside of the casing adjacent to the means (508) to inhibit ingress of moisture or sample.

9. A test device according to claim 8, **characterised in that** the backing material is transparent plastics material.

10. A test device according to any one of the preceding claims, **characterised in that** the porous carrier material is nitrocellulose.

11. A test device according to any one of the preceding claims, **characterised in that** the porous sample receiver protrudes from the casing.

12. A device according to claim 11, **characterised in that** the casing is provided with a removable cap or shroud (503) to protect the protruding porous sample receiver.

13. A test device according to any one of the preceding claims, **characterised in that** it incorporates a control zone (518) downstream from the detection zone (517) in the porous carrier to indicate that the liquid test sample has permeated beyond the. detection zone, the casing being provided with means (509) through which the control zone may be observed.

14. A test device according to any one of the preceding claims, **characterised in that** the analyte is hCG.

15. A test device according to any one of claims 1 to 14, **characterised in that** the analyte is LH.

16. A test device according to any one of claims 1 to 14, **characterised in that** instead of the freely mobile reagent being a specific binding agent for an analyte, the freely mobile reagent can participate in a competition in the presence of an analyte.

Patentansprüche

1. Analytisches Testgerät, umfassend einen trockenen porösen Träger (510), unmarkiertes spezifisches Bindungsreagenz für einen Analyten, welches unmarkierte Reagenz auf dem porösen Träger in einer Nachweiszone (517) permanent immobilisiert und daher in feuchtem Zustand nicht beweglich ist, und in trockenem Zustand stromaufwärts von der Nachweiszone ein markiertes spezifisches Bindungsreagenz für dieselbe Nachweissubstanz, welches markierte spezifische Bindungsreagenz innerhalb des porösen Trägers in feuchtem Zustand frei beweglich ist, so daß die flüssige Testprobe, die möglicherweise die Nachweissubstanz enthält und die auf das Gerät aufgebracht wird, markiertes Reagenz aufnehmen und danach in die Nachweiszone (517) dringen kann, **dadurch gekennzeichnet, daß**

   a) der Markierungsstoff ein teilchenförmiger Direktmarkierungsstoff ist;
   b) der poröse Träger innerhalb des hohlen Gehäuses (500) enthalten ist, das aus feuchtigkeitsundurchlässigem festen Material aufgebaut und mit Mitteln (508) versehen ist, die das Ausmaß (sofern gegeben), bis zu dem das markierte Reagenz in der Nachweiszone (517) gebunden wird, feststellen lassen;
   c) der poröse Träger mit einem porösen Probenaufnehmer (506) verbunden ist und indirekt mit dem Äußeren des hohlen Gehäuses über den porösen Probenaufnehmer in Verbindung steht, auf den die flüssige Testprobe aufgebracht und von dem die aufgebrachte flüssige Testprobe in den porösen Träger dringen kann, und
   d) das markierte spezifische Bindungsreagenz innerhalb des hohlen Gehäuses (500) enthalten ist.

2. Testgerät nach Anspruch 1, **dadurch gekennzeichnet, daß** der poröse Probenaufnehmer aus einem Material mit einer Porosität mit gleicher oder mit mehrfacher Ausrichtung besteht, vorzugsweise aus einem porösen Kunststoffmaterial.

3. Testgerät nach Anspruch 1 oder 2, **dadurch gekennzeichnet, daß** der teilchenförmige Direktmarkierungsstoff ein Farbsol oder ein Goldsol ist.

4. Testgerät nach Anspruch 1 oder 2, **dadurch gekennzeichnet, daß** der teilchenförmige Direktmarkierungsstoff in Form gefärbter Latexteilchen eines maximalen Durchmessers von nicht mehr als etwa 0,5 µm vorliegt.

5. Testgerät nach irgendeinem der vorhergehenden Ansprüche, **dadurch gekennzeichnet, daß** das hohle Gehäuse aus einem opaken oder durchsichtigen Material aufgebaut ist.

6. Testgerät nach irgendeinem der vorhergehenden Ansprüche, **dadurch gekennzeichnet, daß** das hohle Gehäuse aus Kunststoffmaterial geformt ist.

7. Testgerät nach irgendeinem der vorhergehenden Ansprüche, **dadurch gekennzeichnet, daß** der poröse Träger einen Streifen oder eine Folie von porösem Material umfaßt.

8. Testgerät nach Anspruch 7, **dadurch gekennzeichnet, daß** der poröse Träger einen Streifen oder eine Folie von porösem Material umfaßt, der bzw. die mit einer Schicht (511) aus transparentem feuchtigkeitsundurchlässigen Material verstärkt ist, wobei die transparente Schicht in der Nähe der Mittel (508) mit der Innenseite des Gehäuses in Kontakt steht, um den Eintritt von Feuchtigkeit oder Probe zu verhindern.

9. Testgerät nach Anspruch 8, **dadurch gekennzeichnet, daß** das Verstärkungsmaterial ein transparentes Kunststoffmaterial ist.

10. Testgerät nach irgendeinem der vorhergehenden Ansprüche, **dadurch gekennzeichnet, daß** das poröse Trägermaterial Nitrocellulose ist.

11. Testgerät nach irgendeinem der vorhergehenden Ansprüche, **dadurch gekennzeichnet, daß** der poröse Probenaufnehmer aus dem Gehäuse herausragt.

12. Testgerät nach Anspruch 11, **dadurch gekennzeichnet, daß** das Gehäuse mit einer entfernbaren Kappe oder einem Kontaktschutz (503) versehen ist, um den herausragenden porösen Probenaufnehmer zu schützen.

13. Testgerät nach irgendeinem der vorhergehenden Ansprüche, **dadurch gekennzeichnet, daß** es in dem porösen Träger stromabwärts von der Nachweiszone (517) eine Kontrollzone (518) aufweist, um anzuzeigen, daß die flüssige Testprobe über die Nachweiszone hinaus gedrungen ist, wobei das Gehäuse mit Mitteln (509) versehen ist, durch die die Kontrollzone beobachtet werden kann.

14. Testgerät nach irgendeinem der vorhergehenden Ansprüche, **dadurch gekennzeichnet, daß** die Nachweissubstanz hCG ist.

15. Testgerät nach irgendeinem der Ansprüche 1 bis 13, **dadurch gekennzeichnet, daß** die Nachweissubstanz LH ist.

16. Testgerät nach irgendeinem der Ansprüche 1 bis 14, **dadurch gekennzeichnet, daß** das frei bewegliche Reagenz statt als spezifisches Bindungsreagenz für eine Nachweissubstanz in Gegenwart einer Nachweissubstanz an einer Konkurrenzreaktion teilnehmen kann.

Revendications

1. Dispositif de test analytique comprenant un support poreux sec (510), un réactif de liaison spécifique non marqué pour un analyte, réactif non marqué qui est immobilisé de manière permanente dans une zone de détection (517) sur le support poreux et qui n'est donc pas mobile à l'état humide et, à l'état sec en amont de la zone de détection, un réactif de liaison spécifique marqué pour le même analyte, réactif de liaison spécifique marqué qui est librement mobile dans le support poreux lorsqu'il est à l'état humide, de telle sorte qu'un échantillon liquide d'essai suspecté contenir l'analyte et appliqué au dispositif puisse absorber le réactif marqué et ensuite pénétrer dans la zone de détection (517),
caractérisé en ce que

a) le marqueur est un marqueur direct en particules ;
b) le support poreux est présent dans un boîtier creux (500) constitué d'une matière solide imperméable à l'humidité et muni d'un moyen (508) permettant d'observer le degré (s'il en existe un quelconque) auquel le réactif marqué devient lié dans la zone de détection (517) ;
c) le support poreux est lié à un récepteur poreux d'échantillon (506) et communique indirectement avec l'extérieur du boîtier creux par le récepteur poreux d'échantillon auquel l'échantillon liquide d'essai peut être appliqué et à partir duquel l'échantillon liquide d'essai appliqué peut pénétrer dans le support poreux ; et
d) le réactif de liaison spécifique marqué est présent dans le boîtier creux (500).

2. Dispositif de test suivant la revendication 1, **caractérisé en ce que** le récepteur poreux d'échantillon est constitué d'une matière ayant une porosité unidirectionnelle ou multidirectionnelle, de préférence d'une matière plastique poreuse.

3. Dispositif de test suivant la revendication 1 ou la revendication 2, **caractérisé en ce que** le marqueur direct en particules est un sol de colorant ou un sol d'or.

4. Dispositif de test suivant la revendication 1 ou la revendication 2, **caractérisé en ce que** le marqueur direct en particules consiste en particules de latex coloré ayant un diamètre maximal non supérieur à environ 0,5 µm.

5. Dispositif de test suivant l'une quelconque des revendications précédentes, **caractérisé en ce que** le boîtier creux est constitué d'une matière opaque ou translucide.

6. Dispositif de test suivant l'une quelconque des revendications précédentes, **caractérisé en ce que** le boîtier creux est produit par moulage d'une matière plastique.

7. Dispositif de test suivant l'une quelconque des revendications précédentes, **caractérisé en ce que** le support poreux comprend une bande ou feuille de matière poreuse.

8. Dispositif de test suivant la revendication 7, **caractérisé en ce que** le support poreux comprend une bande ou feuille de matière poreuse renforcée avec une couche (511) d'une matière transparente imperméable à l'humidité, la couche transparente étant en contact avec l'intérieur du boîtier adjacent au moyen (508) pour inhiber la pénétration d'humidité ou de l'échantillon.

9. Dispositif de test suivant la revendication 8, **caractérisé en ce que** la matière de renforcement est une matière plastique transparente.

10. Dispositif de test suivant l'une quelconque des revendications précédentes, **caractérisé en ce que** la matière poreuse de support est la nitrocellulose.

11. Dispositif de test suivant l'une quelconque des revendications précédentes, **caractérisé en ce que** le récepteur poreux d'échantillon fait saillie hors du boîtier.

12. Dispositif de test suivant la revendication 11, **caractérisé en ce que** le boîtier est muni d'un capuchon ou d'une enveloppe de protection amovible (503) pour protéger le récepteur poreux d'échantillon en saillie.

13. Dispositif de test suivant l'une quelconque des revendications précédentes, **caractérisé en ce qu'**il renferme une zone de contrôle (518) en aval de la zone de détection (517) dans le support poreux pour indiquer que l'échantillon liquide d'essai a pénétré au-delà de la zone de détection, le boîtier étant muni d'un moyen (509) à travers lequel la zone de contrôle peut être observée.

14. Dispositif de test suivant l'une quelconque des revendications précédentes, **caractérisé en ce que** l'analyte consiste en hCG.

15. Dispositif de test suivant l'une quelconque des revendications 1 à 14, **caractérisé en ce que** l'analyte consiste en LH.

16. Dispositif de test suivant l'une quelconque des revendications 1 à 14, **caractérisé en ce que**, au lieu que le réactif librement mobile consiste en un agent de liaison spécifique pour un analyte, le réactif librement mobile peut participer à une compétition en présence d'un analyte.

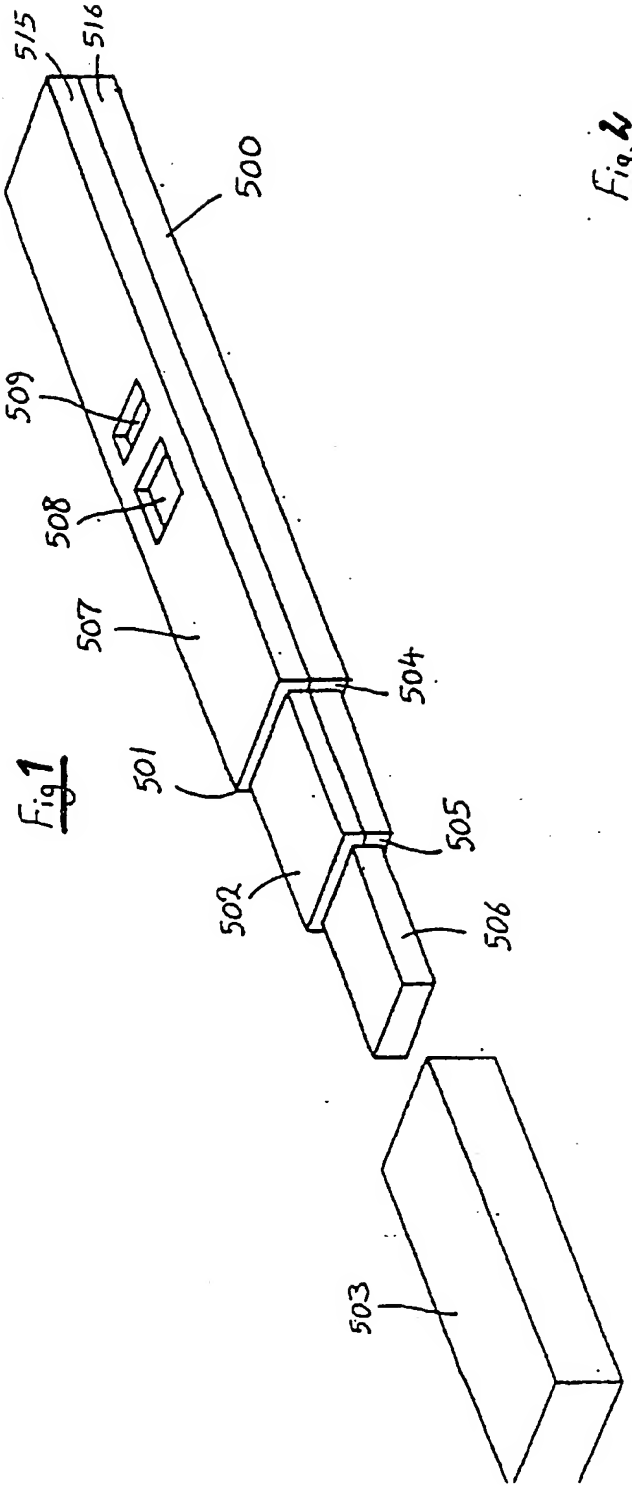
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Fig. 1

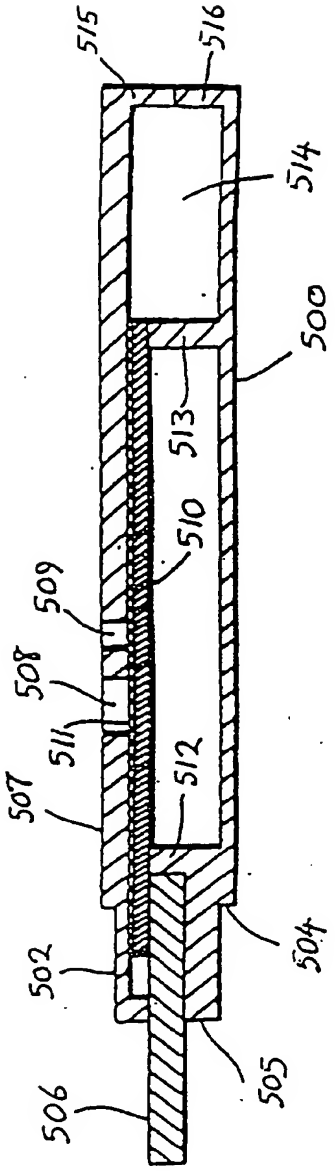
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Fig. 2

Fig. 3

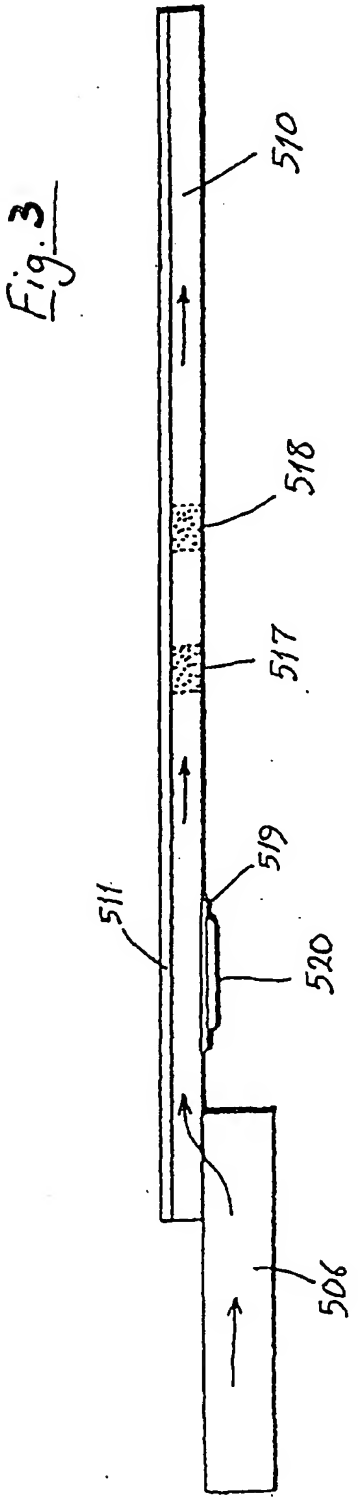

Fig. 4

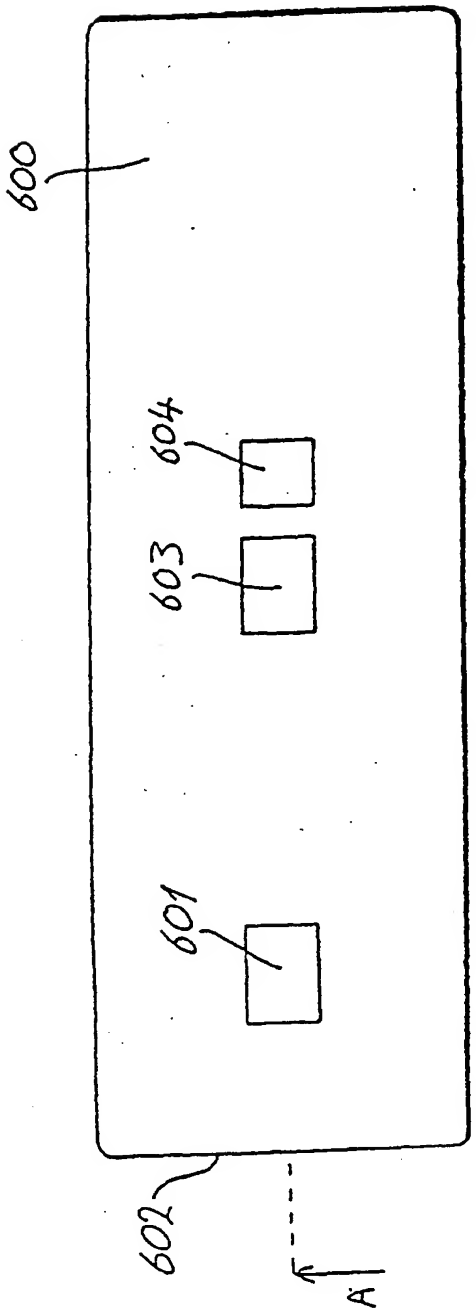

Fig. 5